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Mapping of verticillium wilt resistance genes in cotton

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Abstract

Cotton quality and yield are affected by several factors during the growing season. A soil inhabiting fungus, *Verticillium dahliae* Kleb., can cause substantial yield loss in cotton. A molecular mapping F_2 population derived from the interspecific cross of the highly tolerant *Gossypium barbadense* cv. Pima S-7 and the susceptible *G. hirsutum* cv. Acala 44 was phenotyped for disease incidence and severity. Phenotyping of individual plant reactions to the disease was quantified using a set of growth parameters measured 3 weeks after inoculation. The F_2 phenotypic distribution of these parameters (number of healthy leaves, node number, leaf weight, stem weight, and total shoot weight) suggested that resistance is polygenic inherited. Microsatellites were used to reveal polymorphism between resistant and susceptible parents. A total of 255 simple sequence repeat (SSR) primer pairs were screened over bulks constituted by 10 resistant and 10 susceptible progeny. Sixty markers were used to analyze quantitative trait loci (QTLs). Eleven linkage groups were constructed consisting of 35 markers and spanning 531 cM with an average distance of 15.17 cM. QTL analysis was performed with MapQTL and QTL Cartographer. MapQTL indicated that 15 markers have significant linkage associations and 9 were distributed to chromosomes 10, 11, 12, and 25. Interval mapping also indicated the most likely position of markers that are significant and located on linkage groups. Three loci (CM12, STS1, 3147-2) had large effect on resistance to Verticillium wilt. Two loci were located on LG-1 and one on LG-2 and both linkage groups are located on chromosome 11.

Keywords: Bulk segregant analysis; Cotton; Microsatellites; Quantitative trait loci; Verticillium wilt

1. Introduction

Cotton is the world's leading natural fiber crop that includes about 50 diploid ($2n = 2x = 26$) and allotetraploid ($2n = 4x = 52$) species [1]. There are eight different genome types based on meiotic pairing behaviors in the genus *Gossypium* [2,3]. The allotetraploid species are made up of two subgenomes (A and D) [2,4,5]. With an estimated linkage map of 3700 to 4000 cM [6], approximately 200 framework markers would be needed to provide complete coverage of the genome with an average spacing of fewer than 20 cM. Complete genetic map of the cotton is currently not available.

Despite long-term efforts to use plant resistance and cultural management techniques to control Verticillium wilt in cotton, losses have remained relatively constant for the past 20 years. Modern *G. hirsutum* and *G. barbadense* cultivars show significant variation for important traits including yield, fiber quality, pest resistance, and tolerance to environmental adversities [7,8]. An understanding of the genetic events at the molecular level in this disease interaction will increase our ability to utilize existing

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resistance in cotton germplasm to reduce these losses through conventional breeding.

In many species, bulked segregant analysis (BSA) is used as a rapid procedure for identifying molecular markers in specific regions of the genome, such as genetic markers linked to disease resistance genes [9,10].

Microsatellites have become the molecular marker target sequences of choice for a wide range of applications in genetic mapping and genome analysis [11]. Morgante et al. [12] have shown that microsatellite frequency is higher in transcribed and low copy regions of plant genomes making them more attractive marker class for genetic analysis in plants.

There are many efforts to map genes/quantitative trait loci (QTLs) in economically important traits in cotton for disease and insect resistance, yield, and lint quality [13–24]. The majority of economically important traits including disease resistance can be classified as multigenic. It is often difficult to identify these genes, because the individual effects of each gene on the phenotype may be relatively small. There are many reasons for the inability to recognize individual loci in quantitatively inherited traits. Some disease reactions are difficult to score reliably and others are highly sensitive to environmental factors [25]. Environmentally sensitive traits are difficult to measure accurately, resulting in lowered estimates of heritability and a reduced likelihood for appearing as Mendelian segregation unless experimental precautions are taken. Wilt diseases of cotton, for example, are highly sensitive to environmental differences, especially temperature [26,27]. The environment can affect the expression of wilt symptoms such that under favourable conditions plants that display incomplete dominant resistance may appear recessive [28]. This may also indicate transgressive segregation, where progeny exhibit more extreme resistance or susceptible phenotypes than either parental breeding line, and the dominant inheritance of at least two additively effective factors for resistance [29].

Genetic studies of resistance to Verticillium wilt disease have reported differing inheritance patterns between cultivars of cotton. The interpretation of these differences is complicated by cotton cultivars that, at best, display tolerance to Verticillium wilt disease and not complete resistance [26]. Variations in the methods of disease grading may also contribute to the lack of correlative results regarding the inheritance of disease resistance [26].

Verhalen et al. [30] and Devey and Roose [31] concluded that resistance to Verticillium wilt displayed by *G. hirsutum* cultivars was quantitatively inherited with resistance generally being recessive. Also, Barnes and Staten [32] found that transgressive segregation towards either resistance or susceptibility may occur, and that resistance appears to be quantitative.

Knowing the number of loci influencing the expression of the traits, the location of these loci on the chromosomes and their relative contributions to the phenotypic variation in addition to different interactions among these loci and environment would be very helpful in breeding programs. In the past, classical quantitative trait analysis provided the tools for studying complex disease resistance. However current QTL mapping strategies provide an important means for connecting genome research to plant improvement. QTL mapping has been used to dissect polygenic forms of disease resistance using DNA markers [33].

Recently, molecular markers linked with important traits have been identified in cotton. These include genes for bacterial blight resistance [34] and root-knot nematode resistance (Zhang et al., unpublished data), fiber and seed traits [35], glandless cotton (Decanini et al., unpublished data).

Determining the chromosomal locations of cotton genes/QTL that confer resistance in cotton to *V. dahliae* infection will provide novel basic information, and this will enhance the potential for genetic improvement for resistance to the pathogen in cotton. This study was conducted to determine DNA markers linked to genes/QTLs conferring resistance to wilt disease of cotton. Two bulks, resistant and susceptible to Verticillium wilt, were analyzed to identify markers that distinguish them.

2. Materials and methods

2.1. Cotton genotypes and pathogen isolates

After initial screening of four cotton genotypes with four *V. dahliae* isolates, an interspecific cross was made between highly wilt tolerant Pima S-7 (*G. barbadense*) and susceptible Acala 44 (*G. hirsutum*) cultivars, and F_2 population was screened using isolate V76. The F_1 progeny were grown in the greenhouse to produce F_2 seed and in environmental growth chambers to assay for disease resistance. An F_2 population consisting of 110 individuals was used for mapping. The F_2 plants were derived from a self-pollinated F_1 individual and inoculated with *V. dahliae* isolate V76.

2.2. Inoculation and phenotyping

Acid-delinted seeds of each cultivar were germinated in paper rolls at 30 °C for 24-h incubator and then transferred to 16-ounce plastic cups that were placed in a greenhouse and grown in a soil mixture that was prepared from peat, vermiculite, sand dolomite, maglime, gypsum, and esmigran. After expansion of 5–6 true leaves, the plants were moved to an environmental growth chamber with a 12-h lighted day temperature of 27 °C and dark night temperature of 22 °C. Plants were allowed to equilibrate for at least 1 week at these conditions before inoculation.

Verticillium dahliae isolates were grown on potato dextrose agar plates at room temperature (23 °C) for 3–4 days. For inoculum preparation, a conidial suspension was

spread on plates that were incubated at 25 °C for 3–4 days. Conidia were then collected, washed with sterile water, and diluted to a concentration of 2–5 × 10^7 cells/ml. Plants were stem inoculated immediately below the cotyledonary nodes at two sites with the stem puncture technique using syringe and needle [36]. Plants were incubated under the original growth conditions for 3 weeks post-inoculation and data were collected and disease reactions were scored as described by Devey and Rosielle [37] and Hillocks [38]. The data collected are the number of healthy leaves, number of nodes, leaf weight, stem weight, and total shoot weight. The number of healthy leaves is the number of leaves larger than 2 cm that are fully green and have remain attached on the main stem of the plant. The number of nodes per plant was counted from cotyledonary node to the apex of the plant. Leaves taken off from both the main stem and secondary branches with their petioles attached were used to determine leaf weight. Stem weight was measured by weighing the stem that was cut at the cotyledon node with any leaf or boll material removed. After these measurements, the shoot weight was calculated from the total of leaf weight and stem weight.

2.3. Sampling and DNA extraction

Plant leaf samples were collected from six-week-old plants grown in the greenhouse. Three leaves/plant were harvested from individual F_2 plants and stored at −80 °C until analysis. Genomic DNA was extracted using hot CTAB/PVPP extraction techniques described by Iqbal et al. [39].

2.4. Bulk segregant analysis (BSA)

Phenotypic measurements for each trait were used to score individual F_2 plants and the 10 most resistant and 10 most susceptible plants were chosen for BSA.

After quantification, equimolar amounts of DNA from the 10 F_2 individuals employed to constitute both the tolerant and susceptible pools were prepared and bulked sample were screened over 255 primer pairs. Markers present in both the wilt-tolerant F_2 bulk and Pima S-7 and absent in the wilt-susceptible F_2 bulk and Acala 44 were considered to cosegregate with Verticillium wilt tolerance. Likewise, SSR markers present in both the wilt-susceptible F_2 bulk and Acala 44 absent in the wilt-tolerant F_2 bulk and Pima S-7 were considered to cosegregate with Verticillium wilt susceptibility. Markers that were strong candidates for association with the Verticillium wilt disease response based on chi-square analysis were screened using the entire 110 F_2 individual to map the loci.

2.5. Sources of microsatellites and amplification protocols

The bulks were investigated for any linkage association using microsatellite primers that were obtained from three sources. JESPR primers were developed in a collaborative work between Texas A&M University, and Mississippi State, USDA-ARS [40]. CM primers were developed at Texas A&M University using the techniques described by Connell et al. [41]. BNL primers were originally developed by Benjamin Burr at Brookhaven National Laboratory and are now available at Research Genetics (address: http://www.resgen.com).

Microsatellites were amplified by standard PCR methods [42]. Using loading dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol in water) PCR products were loaded onto gel. Separation of PCR products was performed using agarose and acrylamide gel electrophoresis systems. In the agarose system, a 20-cm long horizontal gel (Owl Scientific) containing 2% low-melting-point agarose plus 2% Metaphor® agarose (FMC) was electrophoresed at 5.3 V/cm in 0.5× TBE buffer with buffer chilling to 4 °C, and stained briefly with ethidium bromide prior to photo documentation. In the acrylamide system, samples were electrophoresed at 20 V/cm in a 10 cm high × 33 cm wide × 1 mm thick vertical gel rig (CBS Scientific) containing 6% polyacrylamide with Spreadex NAB polymer® (Elchrom Scientific) in a 1× TAE buffer, then visualized with ethidium bromide.

2.6. SSR marker analysis on segregating F_2 individuals of the cross between Pima S-7 and Acala 44

One hundred and ten F_2 individuals were screened for the 48 pairs of microsatellite markers. It was expected that the ratio for segregation at a single locus would be 1:2:1. Chi-squared analysis indicates that the candidate SSR markers follow Mendelian expectation (Table 1).

Linkage maps were derived from 48 pairs of markers using Mapmaker® 3.1 [43] (Fig. 1). Markers were grouped with LOD = 3 and three-point analysis was performed at LOD = 4 with 0.40% recombination. Eleven linkage groups (LG-1 to LG-11) were formed consisting of 35 markers and spanning 531 cM at an average distance of 15 cM. Linkage groups were assigned on chromosomes using aneuploid lines. Twenty-five markers did not show any linkage association. Permutation tests were performed with 1000 shuffling for comparison-wise threshold levels separately in different programs at significance levels $P < 0.05$ and $P < 0.01$ [44].

2.7. QTL analysis

Two QTL analysis software programs were used to analyze marker-QTL associations using test statistics with single marker and interval analysis. MapQTL which is used for single marker analysis uses the rank sum test of Kruskal–Wallis [45] based on a nonparametric mapping method to test marker genotypic classes [46]. The test, which is performed on each locus separately, ranks all individuals according to the quantitative trait while it classifies genome contributions according to their marker genotype. QTL cartographer used for interval mapping estimates the

Table 1
Segregation ratios of individual markers having linkage associations with Verticillium wilt resistance with their chi-squares and significance values, in the F_2 population derived from a cross between Pima S-7 and Acala 44

Marker	Linkage groups	*N*	χ^2	*P*	Ratio	Observed segregation frequencies		
						A	H, a_, A_	B
CM12	LG-1	81	17.79	0.0001	1:2:1[b]	28	49	4
CM23	LG-1	83	2.13	0.3447	1:2:1	23	45	15
CM29	LG-1	51	7.11	0.0286	1:2:1[a]	18	16	17
BNL3147-1	LG-3	87	21.55	0	1:2:1[b]	3	56	28
JESPR135-2	LG-3	82	0.8	0.6703	1:2:1	17	43	22
CM50-2	LG-6	90	3.93	0.1402	1:2:1	30	43	17
JESPR270-1	LG-6	93	5.7	0.0578	1:2:1	33	38	22
CM209	LG-9	87	1.48	0.4771	1:2:1	20	49	18
CM50-1	LG-11	88	43	0	1:2:1[b]	45	16	27
CM71-1	LG-11	91	1.43	0.4892	1:2:1	18	50	23
CM71-2	LG-11	93	0.89	0.6408	1:2:1	25	42	26
JESPR291	LG-11	68	5.17	0.0754	1:2:1	25	30	13
CM76	LG-11	86	0.41	0.8146	1:2:1	20	42	24
CM25	LG-11	85	5.4	0.0672	1:2:1	13	52	20
JESPR66	LG-11	90	1.91	0.3848	1:2:1	23	50	17
CM162	LG-11	49	1.69	0.4296	1:2:1	15	20	14

A: homozygous-resistant parent, B: homozygous susceptible parent and H: heterozygous.
[a] Significant at the 0.05 probability level.
[b] Significant at the 0.001 probability level.

probability that a marker is linked to a putative QTL using linear regression model [47–49].

Input files for each program containing the linkage map, and the phenotypic and molecular marker data were prepared according to the instructions given in the manuals [46,50].

The proportion of observed phenotypic variance attributable to a particular QTL was estimated by the coefficient of determination (R^2) from the corresponding model for analysis.

3. Results

Analysis of the F_2 population indicated that Verticillium wilt resistance is a quantitative trait with normal distribution for all traits (Fig. 2). Transgressive segregation towards increased resistance was observed. Binomial curves were shifted towards greater tolerance and indicates a dominant component for resistance to *V. dahliae* infection. Correlations among traits investigated are highly significant at probability level of 0.0001 (Table 2). A total of 255 primer pairs from three sources, JESPR, CM, and BNL, were screened over resistant and susceptible bulks to identify those that are putatively informative. Of 163 JESPR primer pairs screened, 18 were informative in the bulks while 32 out of 83 CM primer pairs were informative for BSA and the BNL microsatellite markers yielded three informative primer pairs out of nine. In total, 48 primer pairs were screened over the entire population. As a result of all screening, 60 markers were scored and analyzed. A total of nine markers were located on chromosomes 10, 11, 12, and 25 based on the traits under investigation.

Single marker analysis of traits that are related to Verticillium wilt resistance revealed significant linkage associations of markers with Verticillium wilt tolerance (Table 3). Using MapQTL, 15 markers were found linked to disease resistance parameters; eight markers were mapped in the Pima S-7 × Acala 44 population.

3.1. Number of healthy leaves (NHL)

Five markers were detected with greater than threshold values for NHL (Table 3). Two markers were mapped on the linkage maps. CM76-2, was found in five traits, explains 14% of the variability, but it was not associated with any linkage group. CM12, CM23, JESPR291, JESPR66, and CM76-2 had highly significant associations. The variability explained by these individual QTLs that are linked to number of healthy leaves ranged from 4 to 13%. Markers, CM12, JESPR291, and CM76-2 had LOD scores grater than 2. An interval mapping approach revealed that, three QTLs were located at positions 6, and 32.9 in LG-1 nearer to CM12 and STS1, respectively (Table 4); one QTL was located in LG-2 in close proximity to BNL3147-2. QTLs located 6 cM from CM12 and 12.10 cM from BNL3147-2 were above the threshold for detection. Variability explained by QTL1 (near CM12) was 17%, and 36% in total in LG-1. QTL3 on LG-2 explained 31% of the phenotypic variability.

3.2. Number of nodes (N)

Eight regions were found to have effects on disease resistance as measured by node numbers (Table 3). Both parents carried QTL alleles that increased phenotypic values. Eight markers, CM12, CM23, BNL3147-1 (located

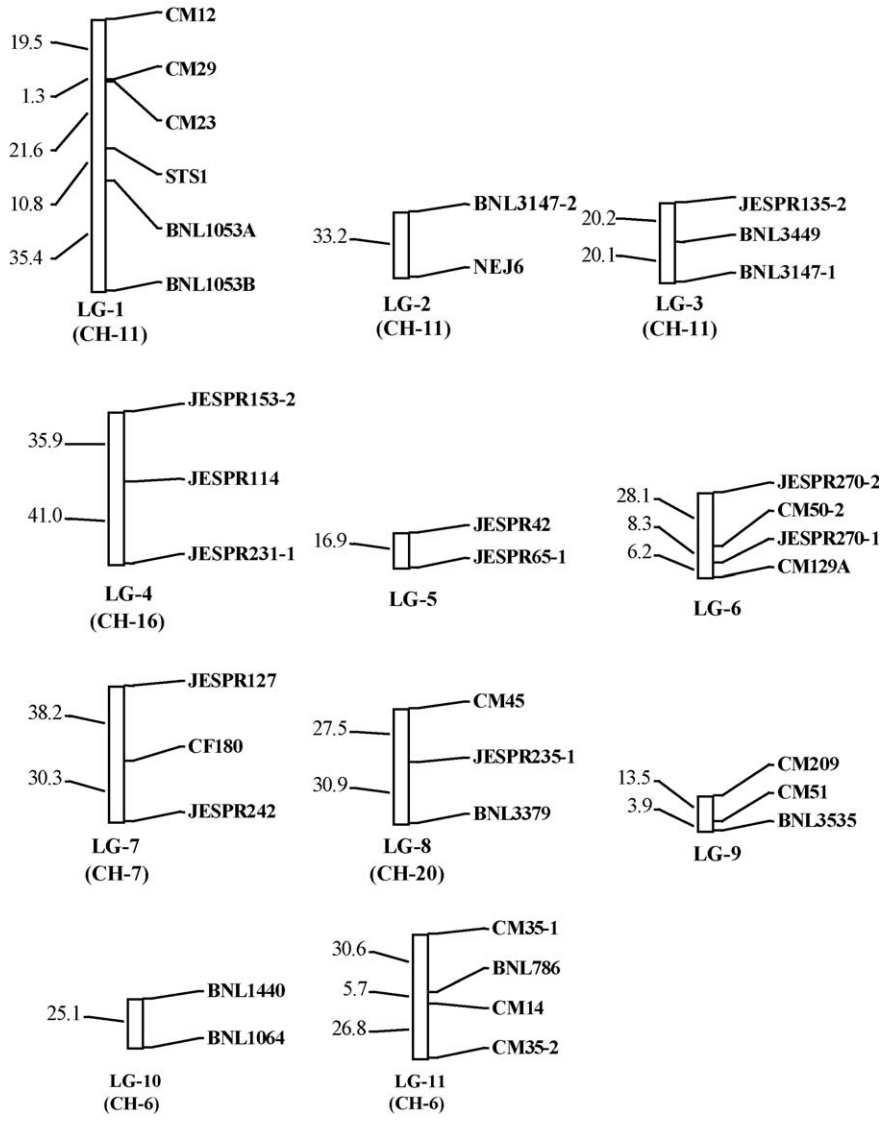

Fig. 1. Linkage groups (LG) consisting of 35 markers including CM, JESPR, and BNL in the Pima S-7 × Acala 44 population identified with Mapmaker 3.1. Software Map distances (cM) indicated in the left and marker names in the right of the map. CH = chromosome.

on chromosome 11), CM71-1 (located on chromosome 10), CM209, JESPR291, CM76-2, and CM 25A were significant with both programs. The marker with the highest phenotypic effect was CM12 (16.93%). CM12 had an LOD of 3.26 while BNL3147-1 and JESPR291 had LOD scores over 2. Interval mapping also revealed some significant associations (Fig. 3). Two QTLs were found over the threshold value, and two had LOD scores of more than 2. QTL1 and QTL2 were located on positions four and 30.9 nearer to CM12 and CM23 (almost same distance from STS1) explained 43% of the variability. One other QTL (LOD > 2) on LG-3 at position 38.2 explaining 13% of the variability (Table 4).

3.3. Leaf weight (LW)

Of the six markers having significant marker–trait associations with leaf weight (Table 3), all were located on chromosomes; CM12 on chromosome 11, CM50-1 on chromosome 12, and CM71-2, JESPR291, CM76-2, and CM162 on chromosome 25. Variability associated with these QTLs ranged from 7 to 18%. CM162 contributed 18% of the phenotypic variability. CM76-2 and CM162 had LOD scores of 2.4 and 2.15, respectively. Interval mapping revealed one QTL on LG-1, located proximal 8 cM from CM12 and explaining 17% of the variability and one on LG-2 at position 20 nearer to NEJ6 and explaining 33% of the variability. Both QTLs were above the threshold values. Another QTL (LOD > 2) was located on LG-4 at position 69.9 and explaining 42% of the variability nearer to JESPR231-1 (Table 4).

3.4. Stem weight (SW)

Seven markers showed significant trait–marker association with stem weight (Table 3). CM29 accounted for 16.4%

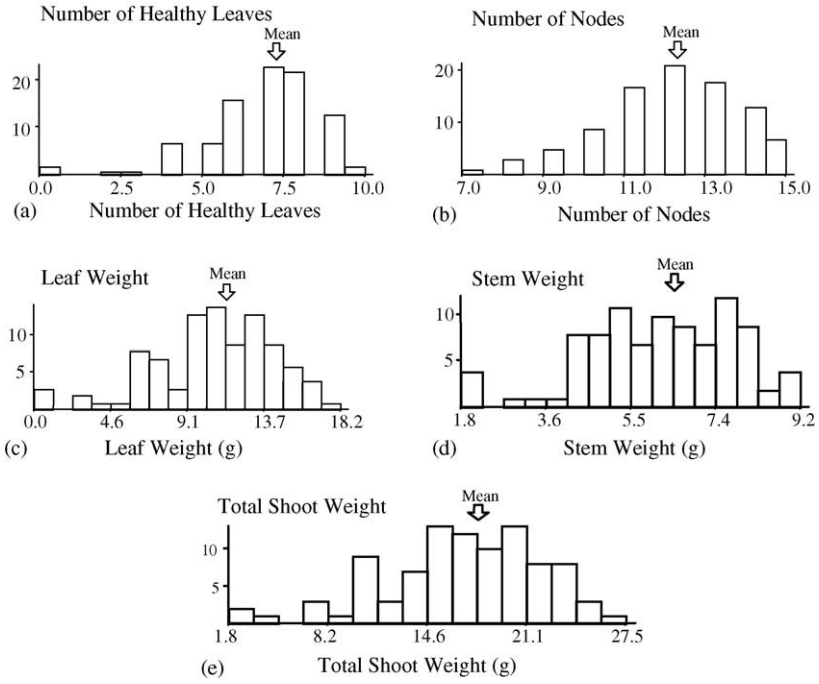

Fig. 2. Frequency distribution of traits associated with response to Verticillium wilt in the F_2 population (Pima S-7 × Acala 44) 3 weeks after inoculation.

Table 2
Correlations among traits associated with resistance to Verticillium wilt in the F_2 population (Pima S-7 × Acala 44)

Cultivar	Number of nodes	Number of healthy leaves	Leaf weight	Stem weight
Number of healthy leaves	0.63			
Leaf weight	0.47	0.84		
Stem weight	0.49	0.64	0.76	
Total shoot weight	0.50	0.82	0.98	0.88

All values are significant at the 0.0001 probability level.

of the variability, followed by CM12 (14.6) and they were located on the same linkage group (LG-1) on chromosome 11. One QTL was detected over threshold value at position 8 nearer to CM12 and explains 30% of the variability. A second QTL (LOD > 2) was located on LG-1 nearer to STS1 (at position 36.9) and explains 17% of the variability. Another QTL (LOD > 2) was located on LG-8 at position 57.5 nearer to BNL3379 and explains 34% of the variability (Table 4).

3.5. Total shoot weight (TSW)

Seven markers had significant trait associations related to disease resistance as measured by the effect of disease on total shoot weight (Table 3). Variability explained by QTLs ranged from 9 to 17%. Three markers, CM12, CM71-2, and CM76-2 had LOD scores over 2. Two QTLs were detected at positions 8 on LG-1 and 20 on LG-2. The first QTL explains 22% and the second 30% of the variability near CM12 and NEJ6, respectively (Table 4).

4. Discussion

Several approaches have been suggested to saturate genomic regions of interest in cotton and other crops with molecular markers. BSA provides a rapid and simple method to identify markers linked to specific genes. The ability to identify markers depends on selecting parental stock with extreme phenotypes associated with the trait.

Out of 255 primer pairs screened, 53 were informative in bulks. Forty-eight primer pairs were screened over the population and yielded 60 markers. It is important when using the BSA strategy to minimize the false-positives and -negatives which are introduced by the populations used, the number if individual in each bulk [51]. False-positive is a problem when sensitivity is high and bulk size is small. For a dominant marker segregating in an F_2 population and unlinked to the target gene, the probability of a bulk of n individuals having a band and a second bulk of equal size not having a band will be $2(1 - [1/4]^n)(1/4)^n$ [9]. Thus the probability of an unlinked locus being polymorphic between bulks of 10 individuals will be 2×10^{-6}. Most of the

Table 3
Single marker analysis in F_2 (Pima S-7 × Acala 44) population to identify marker-QTL association with traits related to Verticillium wilt resistance using MapQTL software

Trait	Linkage groups	Marker	Increased effect	LOD	% Variability	K[a]
Number of healthy leaves						
	LG-1	CM23	Acala	1.3	7.2	8.1*
	LG-1	CM12	Acala	2.5	13.0	9.0*
	LG-2	JESPR66	Pima	0.8	4.1	6.8*
	LG-2	CM76-2	Acala	2.9	14.2	8.2*
	LG-2	JESPR291	Acala	2.0	12.9	8.4*
Number of nodes						
	LG-1	CM12	Acala	3.3	16.9	12.3***
	LG-1	CM23	Acala	1.9	9.9	9.1*
	LG-3	BNL3147-1	Acala	2.1	10.5	9.8*
	LG-9	CM209	Pima	1.4	7.3	6.1*
	LG-9	CM71-1	Pima	1.4	6.7	5.1*
	LG-9	CM76-2	Acala	1.7	7.1	5.3*
	LG-9	CM25A	Acala	1.5	7.6	5.3*
	LG-9	JESPR291	Acala	2.0	12.7	7.6*
Leaf weight						
	LG-1	CM12	Acala	1.8	9.5	6.6*
	LG-7	CM50-1	Acala	1.4	7.2	4.7[a]
	LG-7	CM71-2	Pima	1.9	8.9	6.4*
	LG-7	JESPR291	Acala	1.5	9.5	6.796*
	LG-7	CM162	Acala	2.2	18.3	7.5*
	LG-7	CM76-2	acala	2.4	12.0	7.8*
Stem weight						
	LG-1	CM29	Acala	2.0	16.4	7.7*
	LG-1	CM12	Acala	2.8	14.6	8.7*
	LG-6	JESPR270-1	Pima	1.3	6.4	5.6*
	LG-6	CM50-2	Pima	1.7	8.2	6.9*
	LG-8	CM50-1	Acala	2.3	11.4	10.5**
	LG-8	CM76-2	Acala	1.7	8.5	6.4*
	LG-8	CM71-2	Pima	1.7	8.2	8.1*
Total shoot weight						
	LG-1	CM29	Acala	1.5	12.5	6.4*
	LG-1	CM12	Acala	2.3	12.1	7.6*
	LG-8	JESPR291	Acala	1.5	9.6	6.7*
	LG-8	CM50-1	Acala	1.9	9.3	6.9*
	LG-8	CM76-2	Acala	2.4	12.0	7.0*
	LG-8	CM71-2	Pima	2.0	9.5	7.3*
	LG-8	CM162	Acala	2.0	16.8	7.7*

[a] K = Kruskal–Wallis values.
* Significant at 0.05 probability level.
** Significant at 0.01 probability level.
*** Significant at 0.005 probability level.

markers determined to be positive in BSA are most likely linked to the target loci based on single marker QTL analysis in this experiment. BSA can be used to screen disease resistance to determine linkage association between marker locus and the trait studied.

Many QTL mapping programs and statistical methods, using single marker analysis, interval mapping, and composite interval mapping (CIM) are available; however, all have their limitations and biases. All of these QTL mapping procedures give essentially comparable results unless the heritability of a QTL is very high [52].

Results obtained from single marker and interval-mapping methods showed the same QTL regions having associations with Verticillium wilt resistance. MapQTL uses a nonparametric approach using the Kruskal–Wallis test to analyze marker-QTL association. The power of the Kruskal–Wallis test depends on the degrees of freedom, since it is distributed approximately as a chi-square distribution with the number of genotype classes minus 1 as degrees of freedom. Thus, when co-dominant and dominant loci were combined, the latter may show a smaller significance level even if they are more closely linked. The power also depends on the number of individuals in the test. Differences between markers in number of individuals in the test will affect the gradient in the test statistics over the linkage group. In linkage groups 4 and 8, markers linked to leaf weight and

Table 4
Interval analysis in the F_2 population (Pima S-7 × Acala 44) for the identification of QTLs using QTL-Cartographer

Traits	Linkage groups	Interval over threshold	Max. LR	Position on map	% Explained	Additive effect	Dominance effect	Rt^2
Number of healthy leaves								
	LG-1	2–10	12.78[b]	6	16.97	−0.22861	1.455581	0.1803
	LG-1	24.9–36.9	10.82[b]	32.9	18.85	−0.86962	1.040128	0.1992
	LG-2	14–28	12.10[b]	22.0	31.11	1.5871	2.2101	32.18
Number of nodes								
	LG-1	0–18	15.85[b]	4	19.07	−0.49536	1.245422	0.2013
	LG-1	22.9–36.9	15.10[b]	30.9	24.28	−0.99889	1.011851	0.2534
	LG-1	42.5–50.5	11.35[b]	46.5	16.97	−0.92942	0.695661	0.1804
	LG-1	59.3–69.3	9.99[a]	68	25.61	−0.70138	1.325973	0.2667
	LG-3	36.2–40.2	9.83[a]	38.2	13.45	0.0444	−1.3989	14.52
Leaf weight								
	LG-1	2–18	11.03[b]	8	16.5	−0.1571	2.994086	0.1756
	LG-2	12–28	13.29[b]	20.0	33.22	2.5257	4.7362	34.28
	LG-4	67.9–69.9	9.47[a]	69.9	41.87	3.1256	2.9545	42.94
Stem weight								
	LG-1	0–19.5	18.10[b]	8	29.45	0.01517	1.8193	0.3052
	LG-1	32.9–40.9	10.41[a]	36.9	16.94	−0.8047	0.756008	0.18
	LG-1	42.5–48.5	10.77[a]	46.5	15.14	−0.79628	0.621891	0.162
	LG-8	47.5–57.5	8.99[b]	57.5	33.87	0.2995	1.8824	34.94
Total plant weight								
	LG-1	2–12	14.32[b]	8	21.92	−0.10805	4.777822	0.2298
	LG-2	16–26	10.21[a]	20.0	29.65	3.0212	6.3369	30.72

Rt^2 is the proportion of the total variance explained by the QTL and the background markers and any explanatory variables. LR = likelihood ratio and LR of 9.20 = LOD of 2.

[a] LOD > 2 but smaller than its threshold value.

[b] Significant at 0.05 as a result of 1000 shuffling.

stem weight had high variance explained even though their LOD scores are small. A statistical artifact probably causes this high variance. Also, epistatic interactions can explain very high variability as seen in chromosome 11.

Three loci had large effect on resistance to Verticillium wilt. Two loci were located on LG-1 and one on LG-2. One QTL nearer to CM12 was found in all traits measured. A second QTL that was found in number of healthy leaves, number of nodes, and stem weight was nearer to STS1. There was also a peak between STS1 and BNL1053-1. This was probably not a real QTL because it is very close to another QTL. The third QTL that contributed variations in number of healthy leaves, leaf weight, leaf-stem ratio, and total shoot weight was located between BNL3147-2 and

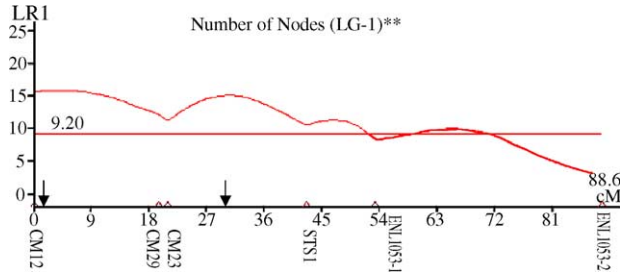

Fig. 3. QTLs that are detected for number of nodes within intervals of markers by interval mapping approach. Arrows indicate the position of individual QTLs. LG is the linkage group. **Above the threshold value.

NEJ6. Another QTL was detected on LG-3 but associated only with number of nodes. Since LG-1, LG-2, and LG-3 were located on chromosome 11, it was concluded that chromosome 11 has a large effect on resistance to Verticillium wilt. In addition, one QTL in LG-4 was linked to leaf weight, and two QTLs on LG-3 and LG-8 were linked to number of nodes and stem weight, respectively. One QTL that is detected in one trait and not detected in another may be due to specificity to one trait.

As pointed out by Paterson et al. [53], studies conducted in a single environment are likely to underestimate the number of QTLs that can influence a certain trait. For such an environment-specific QTL, one would only be able to identify the QTL at a location where these environmental conditions are met. There are also markers having large effects but they were not placed into any linkage group with others. Since 25 markers were not mapped to any linkage groups, it is expected that addition of new markers to the population will improve genome coverage. Because the cotton genome is large, estimated at 4675 cM [54], additional markers will be necessary for sufficient mapping and to saturate the region associated with resistance to Verticillium wilt. This will provide a better understanding of linkage associations for disease resistance at these loci. Twelve markers have distorted segregation at different significant levels including some linked markers; CM12, CM29, BNL3147-1 (mapped), CM50-1, and JESPR135-1

(not mapped). The results showed that markers were located less then 10 cM away from the QTLs on LG-1 (CM12 and STS1), 11–15 cM away from QTL on LG-2 (NEJ6), having strong associations with Verticillium wilt resistance and could prove useful in marker marker-assisted selection but additional markers are needed to locate QTLs in greater probabilities and efficient use. As stated by Stuber et al. [55] marking the entire genome with uniformly distributed loci every 10–20 cM can give a significant increase in the relative effectiveness of marker-assisted selection and QTL identification.

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